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NUTRITION REVIEWS

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NUTRITION REVIEWS

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NUTRITION IN THE SPACE AGE

It appears that the scientist concerned with research in nutrition is about to be charged with the task of solving man's nutrition problems in the milieu of space before he has more than scratched the surface in solving the problems of man in the environment of Earth.

At the time of this writing, there is no question that the age of space flight for man is arriving; but when the age of space travel will arrive is largely dependent on the ability of the scientist to solve the problems concerned with man's physiological needs in space. One may safely say that the engineering problems involved in space flight and space travel are more nearly solved than are the biological problems of the potential astronaut.

In considering space transport it is necessary to differentiate between what is meant by "space flight" and "space travel." Space flight refers to explorations of the area lying between ten and 120 miles above Earth's surface which is called "partial space." Above 120 miles true space begins. It is in the latter area, where transport is interplanetary, lunar or orbital (as a man in a Space Station), that the term "space travel" is used ("Challenge of Man's Entry into Space," Maj. G. A. Champlin, M.C. *U. S. Military Medicine*, **123,** *6 (1958)*). In both areas the biologist is faced with duplicating Earth's environment for the survival of the traveller.

For "space flight" the duration of the trip will be relatively short. For "space travel" the duration of the trip may be days, weeks, months, or even years. The trip to Moon is envisioned as taking three days each way. To Mars it will take perhaps five hundred days each way. It is in the area at and beyond 120 miles that the phenomenon of "zero gravity" becomes a reality, where protection must be afforded against radiation hazards and collision with meteorites, and where there is total darkness.

During the journey to Space, the traveller must take his natural environment with him. He must be afforded protection against the terrific heat generated by air friction as the space vehicle accelerates through atmosphere to the required 25,000 miles per hour needed to escape from Earth's gravitation and enter Space. The traveller must survive a force many times the gravity at Earth's surface during this acceleration. This means subjection to the stress of many G's, which begin to build up from the one G on Earth to X G's when he reaches "escape velocity." The effects of nutrition on recovering from this stress, and resistance to this stress, are largely unknown. The space vehicle must provide food, water, and oxygen and waste products must be removed. The voyager must be in a sealed cabin which must be pressurized from within, since the low density of air in Space precludes compression due to the excessive temperature resulting from compression of rarified air.

Once on Moon or Mars, provision must be made for subsistence and survival. Similarly, a Space station may be built some hundreds of miles above Earth to act as a way station to points further out in Space, and as an observation post to observe all areas of Earth. This space station will be in orbit around Earth just as any man or object will orbit in Space if not propelled.

The problem of pre-travel conditioning nutrition needs further study as Air Force high altitude and G force research has already shown a definite relationship between food intake and physiological response ("Nutrition Research for Man in Space Flight," B. Finkelstein and A. Taylor. Presented at Association of Military Surgeons, November 1958, Washington, D. C.

In press for publication in *U. S. Military Medicine*). Indeed, the Soviets who have sent animals on space flights for a number of years (and recovered them) even before Sputnik II carried the dog into Space, give further evidence of the potential importance of nutrition. In this connection, the immediate effect of meals, *viz.* nutrition, on the cardiovascular and central nervous systems is being studied assiduously in the Soviet Institute of Nutrition ("Some Results of Biological Experiments in Rockets and Sputnik II," Andrei G. Kousnetow. *Journal of Aviation Medicine* **29,** *11 (1958)*).

The dog in Sputnik II was the subject of a true biological experiment on Space travel showing the effects of acceleration and "zero-gravity" on heart contractions, respiration, and the central nerve system (*Ibid.*). After the dog was in orbit in Sputnik II, a return to initial physiological state was observed. The time between the first changes observed during acceleration and the return to the initial state after the dog was in orbit was longer than time previously observed in experiments involving only acceleration with its accompanying noise and vibration (*Ibid.*). This indicated that additional physiological stress was super-imposed at entrance into the zero gravity state. On the other hand, once in zero gravity, will not man's energy requirement decrease since he will no longer combat the stress of gravity experienced by the earth-bound?

Aside from the question of the effect of nutrition on the physiology of the central nervous system, zero gravity further provokes problems of pre-ingestion, ingestion, and metabolic nutrition. At zero gravity everything not fastened down will float, suspended between top and bottom. Liquids will not pour, and in an open container they will tend to break into small particles if the container is moved. This creates a danger if the particles are aspirated.

Solid foods having a tendency to form dust, create another danger in the event the dust particles get into the lungs before the food is put into a semi-liquid consistency by the mouth. Once inside the mouth the food is secured by closing the lips. Chewing and swallowing are no problem.

The logistics of subsistence support for man in the sealed cabin travelling in Space and in provisioning the Space Stations are staggering. Water requirement alone is predicted to be a minimum of five pounds per day per traveller, or approximately a ton a year per traveller. Water, then, becomes a limiting factor in Space explorations. A partial solution will be the development of means to recover, and make safe, water from expired air, perspiration, and the urinary and fecal waste products of the traveller.

For travel in Space or man's existence in a space station, unless "synthetic" gravity can be established in the vehicle, safe feeding can best be done by using liquid or semi-liquid food that can be dispensed via a squeeze bottle or by piping the liquid or semi-liquid food from closed containers directly to the traveller's mouth. Liquid diets high in protein, and adequate in other respects, have been developed at the Air Force Aero Medical Laboratory and are being fed to volunteer subjects. Observations on satiety, gastro-intestinal function, and psychological reactions of men on such a feeding regimen are currently being made at that laboratory (Finkelstein and Taylor, *Loc. cit.*).

Even if the engineer should be successful in creating a "synthetic" gravity in the space vehicle or station, it is unlikely that the conventional foods used in atmospheric flights, because of their bulkiness, will be used for space travel of longer than one or two days duration. Likewise, the conventional heating, storage and preparation equipment have no place in the space vehicle, for they are too wasteful in weight and volume. For space travel, involving days and months, the use of dehydrated foods may provide a partial answer, provided "synthetic" gravity can be induced and the dehydrated food is of the pre-cooked type requiring only the addition of hot or cold

water for preparation. Similarly, irradiated fresh foods, pasteurized to prolong shelf-life to alleviate the need for refrigeration, give promise. Both of these processes, when developed, will contribute immeasurably to the comfort of the traveller and will, if the current trends are indicative, provide wholesome, nutritious, and acceptable foods ("Space Nutrition," J. C. Ebbs. *J. Am. Dietet. Assoc.* **35,** *1 (1959)*).

For interplanetary or lunar travel which would extend, perhaps, for periods of six months to five or six years, some scientists believe that the only source of food logistically feasible is that provided by the use of a closed ecological system (*Ibid.*). Such a system envisions the use of plants to provide food and oxygen to the traveller, and utilization of the traveller's expired carbon dioxide and urinary and fecal products for nourishment and growth of the plants (*Ibid.*). Reports of research in this field indicate that common green algae are particularly suitable.

Five pounds of algae can produce sufficient oxygen to support one man, and will clear a given area of his respired carbon dioxide. Theoretically, a symbiotic relationship between plant and man in a closed system could be established in a sealed cabin. These algae are said to be highly digestible with a proximate composition of 50 per cent protein, 25 per cent fat, 15 per cent carbohydrates, and 10 per cent ash. B vitamins, carotene, and ascorbic acid are also present. Proponents of such a feeding system recognize the need for supplements to provide the lacking essential nutrients.

Aside from the immediate question of the aesthetic acceptance of such an environment and diet even by an eager astronaut, devices will have to be included to separate the growing algae cells from the nutrient media to make the algae safe for consumption and to incapsulate the algae into a container for swallowing. This brings back the ever recurring proposal of providing man his nutrition in a pill, to which is put the counter-question, "is this physiologicaly feasible?"

The absence of light, or, rather, the state of total darkness, which exists in dustless Space is another factor for nutrition research. Physiological effects of living and eating on Earth in the absence of light is being studied at the Air Force Aero Medical Laboratory. Already it has been observed that the food habits and food preferences of some subjects changed under these conditions (Finkelstein and Taylor (*Loc. cit.*)). Some of the subjects snacked at short intervals while others maintained a three-to-four-meal cycle a day. Subjects reported that all meats tasted alike, all canned fruits tasted alike, and that even whole wheat and white bread tasted the same. Probably allied to this are emotional factors. Men who, in high altitude flights, have exceeded 80,000 feet, consistently report a psychological reaction of mixed elation, sorrow, abject loneliness, and remoteness (Champlin, (*Loc. cit.*)). These experiences have been of short duration. The degree to which these emotions increase or subside as deeper entrance into Space is made is unknown.

Considering these thoughts, as well as their implications, a multi-discipline approach does seem to be essential for the solution of the biological problems of man in Space travel. Of these disciplines, nutrition clearly appears to be involved in a fair share of the problems.

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AMINO ACID REQUIREMENTS OF INFANTS

The essential amino acid requirements of infants fed chemically defined diets having a mixture of L-amino acids in the proportions found in human milk have been the subject of long-term studies by workers in the Department of Pediatrics at New

York University College of Medicine. The requirements for threonine (60 mg. per kg. of body weight per day) and phenylalanine (90 mg. per kg. of body weight per day) have previously been determined (*Nutrition Reviews* **14,** *8* (*1956*)).

Using the same technique, S. E. Snyderman, P. M. Norton, D. I. Fowler, and L. E. Holt, Jr. (*Am. J. Dis. Child.* **97,** *175* (*1959*)) have estimated the requirement for lysine. After demonstrating adequate growth and nitrogen retention on the complete diet, lysine was removed and replaced with an equivalent amount of glycine. The lysine intake was studied at various levels. Criteria of adequate intake were growth rates, nitrogen retention, and total serum protein concentrations equivalent to those in the control periods in the same infant. Of the six infants in the study, four received 125 calories per kg. per day and two received 150 calories per kg. per day. The urinary lysine excretion was measured by column chromatography.

The lysine intakes of the six infants that were considered adequate were 87.5, 88.4, 88.5, 90.5, 102, and 103 mg. per kg. per day, respectively. In five of these infants lysine intakes diminished by 10 to 20 mg. per kg. per day proved inadequate by the above criteria. There was a parallelism between the lysine intake and the lysine excretion, but this bore no obvious relation to the nutritional requirement.

The intakes of 90 to 105 mg. of lysine per kg. of body weight per day were compared with those on more natural diets. The determined range compares rather closely with the lysine intakes of 86 to 126 mg. per kg. per day of the infants studied by S. Fomon and C. May (*Nutrition Reviews* **17,** *68* (*1959*)) and were of the same order of magnitude as the lysine intakes of four infants who received formulas containing only four per cent of the calories as protein derived from cow's milk, studied by the present authors. Since adequate nitrogen retention was observed in both of these studies using milk as the source of lysine, they are in agreement with the studies on the synthetic diet.

In a subsequent paper S. E. Snyderman, *et al.* (*Am. J. Dis. Child.* **97,** *186* (*1959*)) have estimated the minimal valine requirement of five normal infants. The technique used was the same as that outlined above, with the exception that three of the infants were fed diets containing corn oil in place of the hydrogenated vegetable fat. The diet was fed at a caloric level of 125 calories per kg. in two infants and 140 calories per kg. in three infants. Intakes of valine considered adequate were 83, 85, 87, 105, and 105 mg. per kg. per day in these infants. Intakes of 64, 85, and 87 mg. per kg. per day were inadequate in three of the infants studied.

There were no specific symptoms attributable to valine deficiency during the 10- to 15-day periods of no valine intake. Anorexia, drowsiness and irritability were noted. Increased urinary excretions of nitrogen characterized the periods of inadequate intake. The excretion of valine was followed in only one subject in which it was quite low during the periods of low valine intake. No consistent trend in the excretion patterns of other amino acids by this subject was noted.

This estimate of valine requirement on synthetic diets of 85 to 105 mg. per kg. per day is slightly higher than the 80 mg. per kg. per day calculated from data on four infants retaining adequate nitrogen when only 4 per cent of the calories were provided in the form of cow's milk protein with supplementation by nonessential nitrogen in the form of either urea or glycine. It was estimated that infants receiving the usual cow's milk formulas would receive at least twice as much valine as required.

The arginine requirement of the infant has also been estimated. S. E. Snyderman, A. Boyer and L. E. Holt, Jr., (*Am. J. Dis. Child.* **97,** *192* (*1959*)) studied three infants aged one week to three and one-half months on synthetic diets identical to those reported above, following removal of arginine and replacement by an equivalent amount of

glycine to maintain a constant nitrogen intake.

In the first infant, weight gain and nitrogen retention were maintained during a period of 35 days without arginine in his diet. In a second infant, weight gain and nitrogen retention were similar to those of the control periods during the deprivation of arginine lasting 28 days. The third patient was a premature infant aged three and one-half months and weighing 4.6 kg. who continued to gain weight and retain nitrogen during a period of 14 days of arginine deprivation.

In comparison with the previous studies, in which the withdrawal of an essential amino acid resulted in arrested growth within 48 hours, the ability of these infants to continue growing without a dietary source of arginine would suggest that they were capable of synthesizing adequate amounts. They further point out that, since spermatogenesis does not occur in infancy, no added load was placed on arginine synthesis in these three male infants. Consequently they conclude that, under the conditions of their study, arginine did not seem to be a dietary essential for the normal growing infant.

These present reports bring to four the individual amino acid requirements that have been determined in normal human infants under six months of age on synthetic diets.

FLUORIDE CONCENTRATIONS IN HUMAN BONES

Previous to the last few years, fluoride analyses of human bones were limited to samples from individuals who suffered from crippling fluorosis. That of H. C. Hodge (Josiah Macy, Jr. Foundation, *Transactions of the 4th Conference on Metabolic Interrelations, p. 250 (1952)*) is the first substantial survey of the level of fluorides to be expected in human bones under circumstances of low fluoride intake in a community with the low level of 0.06 parts per million (ppm) fluorides in the water supply.

Bone samples from about 90 individuals in the first to the tenth decade of life were analyzed. A marked variation among individuals of the same chronological age was observed. In addition there was an increase in fluoride with age from an average of 200 ppm in the first decade of life, to an average of 1250 ppm in the ninth decade.

A necropsy series in Colorado Springs, where the water supply contains 2.5 ppm of fluorides, is of interest in the evaluation of the safety of fluoride ingestion (E. F. Geever, N. C. Leone, P. Geiser and J. Lieberman, *J. Am. Dent. Assoc.* **56,** *499 (1958)*). During the years between 1947 and 1953, 728 necropsies were performed on individuals ten years old or older by the senior author or by resident physicians in training under his direct supervision. There were 188 persons in this series who had lived in Colorado Springs for less than five years, 130 from five to 20 years, and 334 for over 20 years. The remaining 76 persons had lived there for unknown periods.

The available data from these necropsies were classified according to the major cause of death, contributory causes and incidental pathologic conditions within the four groups separated in accordance with length of residence. One out of every four necropsy protocols showed the major cause of death to be neoplasm, a total of 28.8 per cent. Diseases of the cardiovascular system comprised 34.5 per cent.

Certain differences between length of residence and the number of deaths due to cardiovascular conditions and to neoplastic growths were observed which tended to favor the long-residence group slightly with respect to cardiovascular conditions but tended to put the long-residence group somewhat at a disadvantage with respect to neoplastic growths.

The authors stated, justifiably in view of the small size of the individual length of residence and disease groups, that "it would be as unreasonable to attribute an apparent beneficial effect to fluoride in reducing deaths

from cardiovascular conditions in the long term residency group as it would to attribute to it a deleterious effect in elevating deaths from new growths."

In the overall conclusions, the authors stated that the comparative statistical analyses of the pathologic findings revealed no significant differences which could be related to prolonged residence in this environment or to the fluoride content of the water in the community. A further series of necropsies undertaken between 1955 and 1957, with a detailed examination of the bones, is to be reported.

Microscopic examinations were made of 99 bone samples from 37 persons coming to autopsy who had resided ten years or more in communities where the water contained 1 to 4 ppm of naturally occurring, or artificially added, fluoride by E. F. Geever, N. C. Leone, P. Geiser and J. E. Lieberman (*Pub. Health Reports* **73,** *721 (1958)*). Used as controls were 94 bone specimens from 33 individuals who had lived in areas where the drinking water contained less than 0.5 ppm fluoride. The areas of the bones selected in most cases were the body of a lumbar vertebra, a portion of iliac crest and the sixth rib. In addition, the lumbar intervertebral joints were examined for possible changes in the articular cartilage. Occasionally the sternum was also studied.

On the basis of these histological studies, which included a variety of measurements in addition to critical visual examination, no significant differences that could be related to fluoride intake were observed between the fluoride-exposed group and the controls. Microscopic changes in the bones and joints incidental to aging and due to non-fluoride-related conditions, were observed in both series. Nothing was observed comparable to Roholm's findings in chronic fluorosis of calcification of marginal tissues, ligaments and tendons. Focal calcification and cartilaginous changes of the periosteum and adjacent tendon or fascia of the iliac crest were seen with equal frequency and intensity in both the control and fluoride groups, and were interpreted as normal.

Fluoride and ash analyses of the rib, iliac crest, and vertebra were made for 69 persons by I. Zipkin, F. J. McClure, N. C. Leone and W. A. Lee (*Pub. Health Reports* **73,** *732, (1958)*). For ten to 76 years 36 of these individuals had been exposed to 1.0 to 4.0 ppm fluoride. The other 33 had lived in areas with less than 1.0 ppm fluoride for ten to 87 years. Microscopic examinations of the majority of these bones had been made by Geever, *et al.* (*loc. cit.*). Most of the persons whose bones were examined in these studies died suddenly due to trauma, coronary heart disease and cerebrovascular accidents. Bones of six persons with chronic illness or diseases known to affect bone structure, which had been excluded from the microscopic study, were included in the chemical analyses. These cases included three individuals with malignancies and three with renal disease.

The 33 individuals, 27 to 87 years of age, who had lived in areas where the water supply contained 0.1 to 0.4 ppm fluoride had fluoride levels of 410, 500 and 540 ppm for the iliac crest, the rib and the vertebra, respectively. Five individuals 64 to 85 years of age from Grand Rapids, Michigan, after 12 years of fluoridation at 1 ppm had fluoride values of 1380, 1390 and 1600 ppm for the same bones. Twenty-seven individuals from Colorado Springs and Amarillo, Texas, where the water supply contained 2.6 ppm fluoride, had average fluoride values of 2670, 2570 and 2860 ppm, respectively, in the iliac crest, the rib and the vertebra.

In Lubbock, Texas, four individuals had average fluoride values of 4130, 3980 and 4110 ppm, respectively. Some samples of intervertebral cartilage were analyzed and found to contain considerably less fluoride than the bones. There was no evidence from these data to suggest that the degree of calcification of the intervertebral cartilage had been influenced by the different levels of fluoride. These fluoride data, coupled with

the microscopic studies of the preceding paper, constitute substantial evidence that these levels of fluoride may be present in the bones without producing any apparent tissue damage. It is also noteworthy that the six individuals with malignancy and renal disease had fluoride values that were not significantly different from the averages of their groups, in view of variations encountered for the other individuals.

Various bone samples of individuals from Rochester with musculoskeletal disease were analyzed for fluoride (C. L. Steinberg, D. E. Gardner, F. A. Smith and H. C. Hodge, *New Engl. J. Med.* **258,** *322 (1958)*). The samples were obtained at autopsy or in the course of surgical procedures. Eighteen bone samples were analyzed from 14 patients with different forms of arthritis and from one patient who did not have arthritis, all of whom had ingested fluoridated water for periods ranging from three years and five months to four years and seven months. The fluoride levels varied from 915 ppm to 2200 ppm. Fifteen bone samples were analyzed from 11 patients with different forms of arthritis and two patients who did not have arthritis who lived in areas where the water supply contained negligible amounts of fluorides. The fluoride levels varied from 415 ppm for a 21-year-old to 2590 ppm. The average fluoride values for the two groups did not differ significantly from each other.

A careful review of the radiograms of both groups of individuals failed to reveal any relation between various forms of arthritis and the ingestion of fluoridated water at an optimal fluoride concentration. The concentration of fluoride in these individuals fell considerably short of the bone concentrations in persons receiving toxic levels of fluorides over long periods.

Fluoride analyses were made on the cancellous portion of the sixth rib of individuals from Leeds where the drinking water contained virtually no fluorides, and from South Shields and West Hartlepool where the water contained 0.8 and 1.9 ppm of fluorides, respectively (D. Jackson and S. M. Weidmann, *J. Path. Bact.* **76,** *451 (1958)*). The authors indicated that these levels were lower today than ten years ago by undetermined amounts due to recent admixtures with lower fluoride-containing waters. The necropsies from which these samples were obtained were largely on individuals who had died suddenly. Only cases of known bone disease were excluded.

It could not be guaranteed that the individuals had lived for a lifetime in the area in which the necropsy was made, although efforts were made to conform to this requirement. Forty-two samples were available from Leeds representing individuals from 12 to 88 years of age. Twenty-seven samples were available from South Shields representing individuals from 38 to 80 years of age. Fifty-six samples were available from West Hartlepool from individuals from one day of age to 85 years of age. In all three communities, the fluoride concentration in the cancellous bone increased with age until a plateau was reached. In this plateau the average fluoride concentration of the people from Leeds was 1900 ppm, those from South Shields 2450 ppm and those from West Hartlepool was 4000 ppm.

These values are a great deal higher than for comparable individuals from communities in the United States. The authors pointed out that the present samples were from cancellous bone which tends to run about twice as high in fluorides as compact bone. In addition, the authors commented upon the possibility that tea-drinking in England would provide about 1 mg. of fluoride per day to the adult population; this amount would be rarely provided to United States citizens because of their lesser preference for tea.

Chemical analyses of the skeletal tissues of two female septagenarians are reported by F. J. McClure, H. G. McCann, and N. C. Leone (*Pub. Health Reports* **73,** *741 (1958)*). The one, subject A, lived for 24 years, prior to death by heart attack, in Washington,

D. C. where the water contained 0.2 ppm fluoride. The other, subject B, whose death was caused by a cerebral vascular accident, had lived for the preceding 34 years in Bartlett, Texas, where the drinking water contained 8.0 ppm fluoride. The fluoride values of the bones of subject A ranged from 900 ppm for the femur to 1850 ppm for the dorsal vertebra. The fluoride values of the bones of subject B ranged from 7150 ppm for the fibula to 9410 ppm for the rib sample and 9660 ppm for the acromioclavicular joint.

In the latter subject there was a suggestion that there had been a slight increase in the ash and calcium contents of the skeletal tissues. The absence of any gross or systemic findings or of any impairment of health or well-being, malformation of the skeletal tissues or malfunction in the subject who had consumed water containing 8.0 ppm fluoride indicates that human bone is not adversely affected when it contains levels of fluoride in the 7000 to 10,000 ppm range.

These several papers add a great deal to our knowledge in this area of bone metabolism, both in the amount of fluoride that can be expected to be deposited in a low-fluoride area where the daily ingestion from all sources is between 0.3 and 0.6 mg. fluoride, and in the additional amounts of fluoride that can be expected in areas where different levels of fluoride are present in the water supply.

These data again add to the increasing body of information which supports the wisdom and safety of fluoridation at 1.0 to 1.2 ppm in cities in the Northern United States, and at 0.7 to 0.8 ppm in cities in the Southern United States where water consumption is greater because of the higher average temperature.

ESSENTIAL FATTY ACID DEFICIENCY IN INFANTS

It is well known that unsaturated fatty acids (linoleic, linolenic, or arachidonic acid) are essential in the diet of a number of animal species, including rats, chickens, and swine. Studies have been underway for a number of years at the University of Texas School of Medicine, Galveston, to determine the essentiality of fats in the diet of man. A recent report from this laboratory presents clinical manifestations of linoleic acid deficiency in infants who were fed on formulations low in fat (A. E. Hansen *et al.*, *J. Nutrition* **66,** *565 (1958)*).

The infants selected for the study were from families of physicians, medical students, and families seen in the University Well Baby Clinics. Twenty-seven infants received a skim milk formula which supplied 1.4 per cent of the calories as fat (0.1 g. per cent). Linoleic acid was present as 0.1 per cent of the calories (0.04 g. per cent). Protein constituted 15 per cent of the calories, while carbohydrate served as the remaining energy source. Vitamins and iron were added as supplements. The formula contained 63 calories per 100 ml.

The infants also received solid food, beginning with cereals at three months of age; at monthly intervals thereafter fruits, vegetables, and meat were included in the diet. This diet was fed for varying periods, from two weeks to as long as 12 months.

During the study, various fat supplements were also tested. Both trilinolein and natural fat were tested as sources of linoleic acid. Ethyl arachidonate was tried in one infant and tripalmitin in another. Three infants were fed a formula containing only saturated fat.

The infants readily accepted the formula low in fat content, and, in general, weight gains were satisfactory. Some difficulty was encountered with the occurrence of frequent large, dark brown, sirupy bowel movements, and with perianal skin irritations.

Of the 24 infants who were fed the low-fat milk formula for at least a month, 15 developed skin changes. Dryness and leathery thickening, which were readily detectable by inspection and palpation, were characteristic. Some desquamation was also noted as well as annoying exudations in the body folds.

When symptoms of fat deficiency were well advanced, the pediatrician-in-charge added specific fatty acid preparations or natural fats to the diet. In all instances, when the formulation was changed to one containing linoleic acid, the diarrhea stopped, perianal irritation ceased, and the skin gradually returned to a normal soft velvety texture.

At three months of age the blood serum lipids were determined in 16 infants receiving the low-fat milk formula. The concentration of dienoic and trienoic fatty acids was uniformly low whereas tetraenoic acid tended to be high. The average values for di-, tri-, and tetraenoic acids were 3.3, 4.9 and 2.8 per cent of the total serum fatty acids, respectively. Dietary changes which increased the level of linoleic acid consumption (trilinolein, natural fats, cereals or other solid foods) were reflected in the return of serum di- and trienoic acids levels toward normal. Four of the infants were maintained satisfactorily on the low-fat milk formula (plus solid foods as indicated) for as long as six months, and three infants were so maintained for 12 months. The use of solid foods generally resulted in improvement in the low serum levels of the di- and trienoic acids.

Individual case histories are presented. Severe skin changes were induced in a negro infant in ten weeks. The introduction of cereal, in addition to the low-fat milk formula, did not result in any evident improvement in the skin condition. Six weeks later the diet was supplemented with trilinolein to supply 2 per cent of the daily caloric intake. Recovery was prompt and the infant remained normal with the introduction of fruits and vegetables at four months of age.

In another infant, in whom severe skin changes were evident in four weeks, trilinolein equivalent to 2 per cent of caloric intake did not produce any notable improvement. Subsequent introduction of saturated fat (42 per cent of calories) was of no value, but recovery was prompt when the low-fat formula was supplemented with linoleic acid at a level to supply 7.3 per cent of the caloric intake.

Relatively poor response was obtained in the infant whose diet was supplemented with ethyl arachidonate (2 per cent of calories).

From the data presented it is quite clear that young infants are susceptible to essential fatty acid deficiency, and that symptoms may appear within a few weeks if sources of essential fatty acids are withheld. Further study is needed to ascertain whether or not linoleic acid is more effective than arachidonic acid in alleviating symptoms of fat deficiency in man. Arachidonic acid is recognized as the more potent source in studies with rats. The authors point out that the arachidonic acid source used in their studies may have been of questionable purity.

In companion studies the Texas workers also investigated the influence of dietary linoleic acid on caloric intake (D. J. D. Adam, A. E. Hansen, and Hilda F. Wiese, *J. Nutrition* **66,** *555 (1958)*). The 18 infants in this study were fed on varying dietary fat and linoleic acid intakes through modifications of skim milk, half-skim milk, evaporated milk, and special formulations. The linoleic acid level was varied so as to supply from less than 0.1 to as much as 8 per cent of the calories.

The results of these studies suggest an interesting and unique possible metabolic role for linoleic acid. In nearly all instances, when the low-fat formulations were fortified with linoleic acid (as the methyl or ethyl

ester or as the triglyceride), voluntary caloric intake was decreased. Likewise, deletion of dietary linoleic acid tended to produce an increase in caloric intake. Furthermore, the body weight data indicated that dietary linoleic acid was essential for optimum efficient utilization of calories. Specificially, body weight gains were essentially uniform, even though caloric consumption exceeded 125 calories per kilogram of body weight per day on low-fat, low- linoleic acid formulations, while caloric consumption on linolate-supplemented formulations was generally less than 125 calories per kilogram of body weight per day.

The results substantiate the tentative conclusion that, for optimum utilization of energy, the human infant probably needs to consume about 4 per cent of the total calories as linoleic acid. This appears to be within the range of linoleic acid found in breast milk, namely 4 to 5 per cent of the calories.

ANOREXIA NERVOSA—PSYCHO OR SOMATIC

One of the earliest descriptions of anorexia nervosa has been attributed to W. W. Gull (*Trans. Clin. Soc. Lond.* **7,** *22 (1874)*). This disease, which is typified by a lack of appetite (anorexia), was described by Gull as due to a "morbid mental state." His patients "drifted their own way into a state of extreme exhaustion." J. A. Ryle (*Lancet* **2,** *893 (1936)*) believed that a psychic trauma or foolish habit led to a loss of appetite which resulted in starvation, followed by an aggravation of the anorexia. Ryle further asserted that in the physician's approach to the patient, history taking had been replaced by psychoanalysis and physical findings ignored.

E. Williams (*Brit. Med. J.* **2,** *190 (1958)*) has re-emphasized a vigorous somatic approach to the disease. He does not discard the possibility that a psychiatric disturbance may exist. Nevertheless, he considers anorexia nervosa as a wasting disease from which a number of patients die as a result of malnutrition. For this reason Williams believes that "renourishment" should be the first object of treatment. In the report cited above he describes the treatment of 53 female hospital patients admitted with this disease over a period of 56 years.

The patients ranged in age from 12 to 36 years, had amenorrhea and severe weight loss, but no evidence of primary organic disease.

Only a few of the patients were confined to bed and special diets were not prescribed in most cases. Seven of the patients were tube fed and all of them gained weight, averaging 22 pounds per patient. One of these died four years later, emphasizing the importance of preventing relapse by maintaining close liaison with the family doctor and others closely connected with the patient's care. Of the remaining patients, seven gained more than 14 pounds while in the hospital. Seven patients continued to lose weight. Follow-up studies were done in 42 out of 49 patients admitted before 1954. Ten died, two of these from pulmonary tuberculosis, seven from inanition, and one from an unrecorded cause.

In 23 cases, followed for three to 21 years, recovery was complete; 12 had married and 6 of these had children. Six patients were reviewed on an average of nine years after admission. Five remained underweight and retained neurotic symptoms, although they were engaged in regular work. One regained normal weight. Three patients did not improve. Of these, one remained indoors and poorly nourished, one was an hysteric and one may have died.

The author, E. Williams, observed that the outcome in this series of patients was little affected by hospital treatment. Most of the patients questioned could not give a reason for their recovery. He emphasized,

however, that the high mortality justified a vigorous attempt to treat the malnutrition.

Psychiatrists have considered psychiatry as the most appropriate treatment. F. S. Dubos (*Am. J. Psych.* **106,** *107 (1949)*), for example, stated, "It is the neurosis and not the appetite that must be the prime focus of attack by the physician. Therefore, psychotherapy is the major remedial agent. Little reference should be made to diet, and the family and nursing personnel are instructed to avoid talking about food or the patient's dietary habits." Nevertheless, Dubos' patients were not cured. Of the ten reported, five remained thin and unhappy, two defaulted and three continued treatment.

The tendency for current views on treatment to disregard the somatic aspect of anorexia nervosa may be partly due to the "discouraging results of treatment based on general medical principles" in the opinion of E. Williams. He believes that the tendency of the disease to simulate the psychoses may have given physicians the feeling that it was beyond their ability to treat. Nevertheless, the appearance of neurotic symptoms and depression are not enough to suggest the beginnings of psychosis. In fact, B. C. Schiele and J. Brozek (*Psychosom. Med.* **10,** *31 (1948)*) believe they may often be the direct result of inanition.

A. Hurst (*Proc. Roy. Soc. Med.* **32,** *744 (1939)*) claimed that explanation, firmness, and good temper would persuade the most resistant patient to eat a full meal, and that, after that, progress would be continuous. Unfortunately the Hurst method has been ineffective in the hands of others.

The tube feeding used in seven patients described by E. Williams resulted in an average weight gain of 22 pounds. Of the 44 patients not tube fed, the average gain was 9 pounds, with only seven gaining more than 14 pounds. One advantage of the tube feeding method mentioned by the author is that the initial resistance to this procedure is less than to normal feeding, and, further, that the drawn out ordeal of each meal is avoided. Since caloric intake can be adjusted to exceed the requirement, the rate of recovery will be maximal.

In some patients no treatment is tolerated or successful. Favorable responses following lobotomy have been recorded in some recalcitrant patients (P. E. Sifneos, *Am. J. Psychiat.* **109,** *356 (1952)*). Refractoriness cannot serve as a single criterion for recommending this procedure since two patients in the present series recovered after refractory periods of 8 and 11 years.

Summary: Analysis of 53 cases of anorexia nervosa revealed that tube-feeding benefited the patients thus treated. Of ten patients that died, death resulted from malnutrition in eight. Twenty-three recovered spontaneously. It is to be hoped that long term study of the tube-fed patients and suitable control individuals may indicate whether lasting superior benefit will result from this type of active "renourishment."

MINAMATA DISEASE

During the past few years the inhabitants of the Island of Kyushu have been afflicted with an apparently new disease resulting from the ingestion of fish or shellfish. D. McAlpine and S. Araki from the Department of Neuropsychiatry, Kumamoto University, Japan, found 56 people with this new type of neurological disease which was named Minamata disease for the industrial town near which all of the patients resided (*Lancet* **II,** *629 (1958)*). The first symptoms appeared in the patients toward the end of 1953. The number afflicted with the disease increased through 1956, when a governmental edict was passed to prohibit fishing in the bay. Since then, no new cases have been reported.

Minamata, the chief industrial town in the

afflicted area, is the site of a large artificial fertilizer factory. Prior to 1950, the effluent from the factory was poured into the sea. At the latter date, a new sewer line was built which poured the water into Minamata Bay. It was not until three years later that the first neurological disorders were seen.

The first symptom noticed was a numbness in the extremities and sometimes around the mouth, frequently followed by slurred speech, unsteady gait, and increasing disability. Deafness and disturbances of vision, the latter being confined to constriction of the visual field, were seen in most cases. Difficulty in speech, insomnia, and emotional disturbances were seen in many of the patients. Mental confusion and muscular incoordination occurred in all the patients. The more seriously ill adult patients showed an increased amount of amino acids in the urine and increased levels of gamma globulin in the blood.

The most seriously ill died; the mortality rate was 33 per cent. Eight patients died within two months after showing the first symptoms, while four died within the first year. The other patients remained incapacitated to varying degrees. There apparently was no recovery once the individual was afflicted with Minamata disease.

Four of the hospitalized patients were autopsied. They showed small necrotic areas in the intestinal mucosal membrane as the only abnormality in the viscera. The central nervous system showed cellular degeneration which was most marked in the granular layer of the cerebellum; there was dilatation of the perivascular spaces, with occasional softening, but no demyelination. There was edema and occasional small hemorrhages. The spinal cord showed only minor changes.

All 56 patients who had Minamata disease frequently ate fish from the bay. In many instances, two or more members of the same family had the disease. In the neighboring families where this disease did not appear, fish from the local bay was consumed either not at all or in very small amounts. The affected individuals ranged in age from a few years through adulthood, with all ages and both sexes being equally represented. Infants were protected, probably because they were breast-fed until the age of two.

The factor causing the neurological disturbances is unknown. It appears to be heat stable, since some of the individuals who developed the disease ate nothing but cooked fish. Cats, crows, and possibly such animals as pigs and dogs, appeared to be susceptible to a similar disease. The brains of ten cats that had died of this condition showed changes similar to those seen in the human material. It was reported that the disease could be readily produced by feeding cats fish from the bay for two to four weeks.

Although the fish from the bay and the effluent from the factory showed increased amounts of manganese, selenium and copper, the manifestations of the disease are not those normally associated with the toxicity of these elements (*Nutrition Reviews* **3,** *50 (1945)*).

McAlpine and Araki point out that the degeneration of the cerebellum which is a conspicuous feature of Minamata disease is non-specific. This condition is found in a number of pathological states such as anoxia, hyperglycemia, hypoglycemia, mercury intoxication and chronic alcoholism. They suggest further that the symptoms associated with thallium poisoning approach most closely those of Minamata disease. Although the amount of thallium in the mud secured from Minamata Bay was increased when compared with the analysis of sand from another part of the coast, there was, however, no increase in the thallium content of the fish and shellfish from the bay.

The disease is of interest since it points out once more that under certain circumstances toxic materials apart from those of microbiological origin may be transmitted through food (*Nutrition Reviews* **2,** *97 (1944)*). The toxic substance in the fish from Minamata

Bay has apparently no, or at most a minor, influence on the fish themselves. It does, however, produce an irreparable neurological disorder in human beings of all ages. The long latent period (three years from the change in the sewer lines until the first symptoms appeared) and the prompt cessation of new cases when fishing in the bay was prohibited, are intriguing. This suggests that perhaps some initial change occurred in the fish prior to their becoming toxic and that this change was a rather slow one, or that some change occurred in the manufacturing process just prior to the first appearance of the disease.

The present report is an English summary of the current status of the Japanese work on this disease. There appears to be a large amount of biological and biochemical work which should be done before the factor responsible for Minamata disease can be recognized.

THE EFFECTS OF PROTEIN MALNUTRITION ON SKELETAL MATURATION

The skeleton provides a sensitive index to long-term effects of metabolic and nutritional disease. With the use of suitable standards (*Nutrition Reviews* **16,** *4, 101 (1958)*) fairly accurate assessments of skeletal age can be made from X-ray films that have been made by appropriate techniques.

Important criteria for determining skeletal maturation are not only the appearance of ossification centers, but also the shape and degree of calcification of the growing portion of bones. Standards for evaluating the maturation of the knee have recently been published by S. I. Pyle and N. L. Hoerr (*Radiographic Atlas of Skeletal Development of the Knee, Springfield, 1957, Charles C Thomas*).

P. R. M. Jones and R. F. A. Dean (*J. Pediat.* **54,** *176 (1959)*) have used this atlas to study the developmental stage of the bones of the knee in children with kwashiorkor (protein malnutrition). In order to evaluate possible differences between Cleveland, Ohio and Kampala, Uganda, children, 23 boys and 24 girls varying in age from 8 to 38 months were studied. These healthy children were members of the families of the African staff of the Agricultural Research Station near Kampala and were all of the Ganda tribe. They had all been under medical supervision most of their lives and came from economically privileged families who had access to cow's milk, as well as food of all kinds.

Seventy-five children, 44 boys and 31 girls, suffering from severe kwashiorkor, were studied as they entered the hospital. They all had growth failure, edema and hair changes and almost all had marked skin changes. Their ages were not accurately known, but were believed to be accurate within one month.

The knees of the boys in the healthy group were nearly at the stage of development usual in American boys of the same age. The girls, however, were mostly a little in advance of their American counterparts, but the number of children was too small for a proper statistical analysis of the difference. There was retardation in bony development in both boys and girls with kwashiorkor. This retardation was so great that there was almost no overlapping between the healthy and the malnourished groups and consequently the difference did not need to be subjected to statistical treatment. In almost every child the knee was apparently more retarded than the hand which was surveyed in these children as well as in a previous group (*Jones & Dean, J. Trop. Pediat.* **2,** *51 (1956)*).

Compared with the healthy children, the bones of the children with protein malnutrition were smaller, less well calcified and had a diminished trabecular pattern. They

also showed several abnormalities: thinning of the cortex of the shafts of the long bones of the leg; faintness of the zones of provisional calcification at the metaphyses; growth arrest lines; and irregularity of calcification of the edges of the epiphyses. These abnormalities were present in between 57 and 95 per cent of the patients.

There was a high degree of correlation between the degree of retardation in skeletal development and measurements of height or subischial length. An examination of the knee might replace these observations as an assessment of growth retardation. Although no single abnormality found in the knees of children with kwashiorkor could be considered diagnostic, the abnormalities together made a striking picture that the authors believed had not been described in any other pathological condition. The degree of retardation was much greater than could be accounted for by the known length of the period of ill health and it seemed obvious that a record of the length of the period of malnutrition was recorded in the growth retardation of the long bones. The transverse lines believed to indicate disturbed growth at a period in the recent past may have no more meaning than suggesting a general tendency of such children to succumb easily to minor illnesses.

This study demonstrates quite conclusively that protein malnutrition, at least in its severe forms, profoundly influences both the rate and character of bone growth. These changes are most pronounced in the knee. The observations confirm the clinical impression that the appearance of the edema in these patients is only the end result of a longer period of deprivation, interspersed with bouts of intercurrent illness.

THE DESTRUCTION OF VITAMIN A BY BLOOD

Relatively little is known about the ultimate disposition of most vitamins. A small percentage of the daily intake of the water-soluble vitamins appears in the urine and a still smaller percentage is stored in various tissues. There is, however, a large fraction of the daily intake which cannot be accounted for by any technique. Still less is known about the excretion and degradation of the fat-soluble vitamins. Although it has been recognized that the body destroys large amounts of the ingested vitamins, relatively little experimental evidence has been available on this point.

The work of S. K. Kon, W. A. McGillivray and S. Y. Thompson at the National Institute for Research in Dairying, Reading, England (*Brit. J. Nutrition* **9**, *244 (1955)*) showed that large amounts of vitamin A and carotene are rapidly destroyed by the body within a short period after their injection. When 1 mg. of carotene suspended in a solution of "Tween" was injected intravenously into rabbits, the level of carotene in the blood five minutes later was such that it could account for all of the carotene. Two hours later the blood level accounted for approximately 6 per cent of the injected dose, and after the sixth hour practically none of the carotene could be accounted for in the blood.

Of the carotene injected into the rabbits, only a small amount was found in the heart, lungs and kidneys. In these organs the carotene could be detected through the third hour, but after that, it was no longer detectable. The liver contained about ten times more of the compound than the previous organs, but by the third hour it had less than 5 per cent of the injected dose. Some of the carotene was converted to vitamin A, but this accounted for only about 25 per cent of the carotene.

When two mg. of vitamin A acetate suspended in "Tween" were injected into the rabbits, only about 20 per cent of the dose

could be detected in the blood, heart, lungs, kidneys and liver five minutes after injection. Carcass analyses (excluding blood, heart, lungs, kidneys and liver) showed that one hour after injection, 30 per cent of the vitamin was still present. This had been reduced to 10 per cent at the end of three hours. These results led the investigators to conclude that "the injected vitamin A must in fact have been destroyed." The injection of "Tween" itself had no such effect on the vitamin A normally present in the blood.

An interesting observation of the above work was the rapid de-esterification of the acetate by the blood. Within 30 seconds after starting to inject vitamin A acetate, a sample of blood taken from another part of the rabbit showed that approximately half of the injected ester had been converted to the alcohol.

Similar experiments with rats also indicated a rapid disappearance of both vitamin A and carotene. These experiments were not reported in sufficient detail to permit calculations of the percentages disappearing at various intervals from the bodies of animals.

The above work was extended by C. J. Pollard and J. G. Bieri at the National Institutes of Health at Bethesda (*Brit. J. Nutrition* **12,** *359 (1958)*). They checked the recovery of vitamin A suspended in "Tween" when added to a homogenate of a whole rat. Under these conditions, they could recover 95 per cent of the added vitamin A. This finding strengthened their observation that vitamin A injected intravenously into vitamin A-deficient rats was rapidly destroyed. Less than five minutes after injection of 130 micrograms of vitamin A into each of two such rats, they recovered only 64 and 44 per cent, respectively, of the injected vitamin in the homogenate of the entire carcass.

To determine which organ was responsible for the rapid destruction of the vitamin A, they incubated 100 micrograms of the vitamin with homogenates of liver, kidney, lung and spleen. The vitamin was relatively stable in all homogenates. However, when 100 micrograms of vitamin A acetate was incubated with 6 ml. of hemolyzed whole blood, 46 per cent of the vitamin was destroyed at the end of two hours. Washed red cells (hemolyzed) destroyed practically all of the vitamin A, whereas the serum was inactive.

The above work by Pollard and Bieri (*loc. cit.*) was carried out with rats less than 60 days of age. When older rats were used, the red blood cells destroyed much less of the vitamin during incubation. There was an inverse correlation between the age of the rats and the degree of destruction of incubated vitamin A. With normal rats, destruction ranged from 91 per cent for 14-day-old rats to 8 per cent for those over 80 days of age. There appeared to be a break in the curve about the fifth week of age. This observation suggested that the reticulocytes which are known to be present in large amounts in the blood of young animals might be responsible for the destruction of the vitamin.

To test this hypothesis, reticulocytosis was induced in 70 to 80-day-old rats by injecting phenylhydrazine. Eight days after injecting rats with phenylhydrazine, the blood from these rats destroyed 82 per cent of the vitamin A added to it in the *in vitro* test. Blood from the control, non-injected animal showed 20 per cent destruction under similar circumstances. Reticulocyte counts made on the blood of the phenylhydrazine-injected rats showed that there was no direct correlation between the number of these cells and the amount of vitamin A destroyed during incubation.

To rule out the possibility that phenylhydrazine itself was involved in the destruction, the investigators produced reticulocytosis by repeated bleeding of mature rats. Under these circumstances, the vitamin A destructive activity of the blood increased with the accompanying reticulocytosis.

Reticulocyte counts were made on normal rats starting at age 21 days. These rats showed a gradual decrease in the vitamin A destructive activity of the blood through the thirty-fifth day of life. After that age, the blood destroyed approximately 25 per cent of the vitamin A incubated with it. Again, there was no direct correlation between the activity and the level of reticulocytes.

Similar studies made with the rabbit showed that blood from 14-day-old animals had great activity, but this had largely disappeared by the time the rabbits were 35 days old. Blood from 14-day-old mice destroyed 20 per cent of the vitamin A, whereas that from 21-day-old mice destroyed only 4 per cent. Blood from an eight-day-old guinea pig destroyed 12 per cent of the vitamin A. Blood from chicks ranging in age from one to 28 days destroyed none of the vitamin A.

Studies with rats indicated that both vitamin A acetate and the alcohol were destroyed at approximately the same rate. The palmitate ester and natural esters of vitamin A from fish oil were considerably more resistant than the acetate or vitamin A alcohol when incubated with rat's blood. Preliminary studies with carotene indicated that its rate of destruction on incubation with hemolyzed whole blood was similar to that of vitamin A.

Pollard and Bieri (*loc. cit.*) theorized that substances such as unsaturated fatty acids and peroxides, which have been reported by S. Rapoport, W. Gerischer-Mothes and C. Nieradt (*Hoppe-Seyl. Zeit.* **300,** *174 (1955)*) to be present in increased amounts in the blood of rabbits made anemic by injections of phenylhydrazine, might be responsible for the observed effects. The above compounds may not be the only factors involved in the destructive activity of blood, since Pollard and Bieri (*loc. cit.*) reported that preliminary studies indicate differences between the action of the blood from young rats and the blood of the anemic rabbits used by Rapoport *et al.* (*loc. cit.*).

The significance of the above observations remains to be elucidated. The investigators point out, however, that they know of no observations suggesting that blood vitamin A is lowered in conditions associated with reticulocytosis such as occurs in hemolytic anemia.

The work of both the English and the American investigators shows that when either vitamin A or carotene is injected into a number of different species of animals, these compounds are rapidly destroyed by the body. Blood appears to be the tissue primarily responsible for the destructive action. In those animals that show a rapid rate of vitamin A destruction, the rate is faster in the blood of those animals having a high reticulocyte count. The intriguing observation is that the vitamin A-depleted young rat shows the same high rate of destruction as the normal animal. It would be assumed on purely teleological grounds that the deficient animal would conserve all of the available vitamin.

METABOLIC INFLUENCES ON WOUND HEALING

During the past decade many important advances have been made in our knowledge of the growth and metabolism of connective tissue. As Dunphy (*Surg. Gynec. & Obstet.* **102,** *750 (1956)*) has pointed out, connective tissue can no longer be considered an inert supporting substance, but must be looked upon as a tissue which undergoes change in response to hormones, metabolic alterations and a great many disease states. Surgeons have been particularly interested in the possible practical applications of our increased knowledge regarding the growth of connective tissue in the realm of wound healing. Recent studies have been directed toward the various factors which may promote or

delay the healing of wounds as measured by the tensile strength in the post-operative period.

By making chemical analyses of the growing granulation tissue at different stages of healing, Dunphy and Udupa (*New Engl. J. Med.* **253,** *847 (1955)*) demonstrated a "normal pattern" of wound healing. In the early phase which lasts for about five days, mucopolysaccharides and soluble protein precursors of collagen are produced. The second phase is the collagen phase in which normal collagen fibers are formed. The tensile strength of wounds has been shown to rise during the second phase in parallel with collagen formation. These same authors showed that by depriving animals of vitamin C, the first phase was prolonged, but the synthesis of collagen was prevented. By feeding animals a protein deficient diet for a period of five days before wounding, they demonstrated that the mucopolysaccharide state is again prolonged, but that the peak levels are below normal and that the synthesis of collagen is retarded. By simply adding methionine to the protein-free diet, this picture could be almost completely corrected.

Both these and similar experiments, as well as observations of innumerable post-operative patients, have indicated the importance of the general state of nutrition in influencing wound healing. Many factors which might promote healing by virtue of their general anabolic effect, have been studied. Among the most important of these are the hormonal factors.

In a recent paper Prudden, Nishihara, and Ocampo (*Surg. Gynec. & Obstet.* **107,** *481 (1958)*) compare wound healing in a group of rats treated with growth hormone versus that in an untreated group. In each instance the experimental animal was given 4 mg. of bovine growth hormone subcutaneously for seven days. The tensile strength of the wounds of the treated animal averaged 8.8 per cent greater than the control group. The authors attributed this increase in wound strength to the general anabolic effect of the growth hormone as evidenced by a mean weight gain in the experimental animals of 9.1 g., as compared with a mean weight loss in the control animals of 6.9 g.

This work conflicts with the earlier studies of Cuthburtson and co-workers (*J. Endocrinol.* **2,** *475 (1941)*) who found no acceleration of wound healing in rats treated with crude anterior pituitary extract despite a significant anabolic effect in the animals as evidenced by weight gain. They did not, however, measure the tensile strength of the wounds.

There has been no recent experimental work on the effect of androgen on wound healing although the anabolic effect of testosterone and related compounds is well recognized. Among the many studies on nitrogen metabolism in the postoperative patient is the work of Ohin, *et al.* (*Arch. Surg.* **75,** *629 (1957)*). These authors studied the effect of norethandrolone (17 α-ethyl-17 hydroxynolandrosterone; "Nilevar") in a group of well nourished patients who underwent elective abdominal hysterectomy. Each patient in the experimental group was given a daily intramuscular injection of 25 mg. to 50 mg. of this steroid and was in significantly positive nitrogen balance as compared with a negative balance in the control group.

Among the studies of hormonal effects in wound healing should be cited the work of Moltke with thyroxine (*Acta. Endocrinol.* **24,** *229 (1957)*). In his earlier work he showed that D,L-thyroxine reduces the tensile strength of healing wounds in guinea pigs, perhaps by inhibiting one of the factors important in healing. In this more recent study, he demonstrated that D-thyroxine produced an increase in the tensile strength of healing wounds in guinea pigs. In view of his earlier work with D,L-thyroxine, this effect of the D-isomer is attributed to an inhibition of hormone release from the thyroid gland by depressing the pituitary gland.

He further concludes that the inhibitory effect on wound healing by D,L-thyroxine must be due to a peripheral action.

The retardation of epidermal regeneration, capillary proliferation and the formation of granulation tissue by cortisone and related steroids is well known and need not be reiterated. Edwards and Dunphy have summarized the effects of the adrenal steroids and ACTH in a recent review (*New Engl. J. Med.* **259,** *275 (1958)*).

Despite all these recent studies of the effects, both beneficial and deleterious, of various agents on wound healing, it is generally accepted that there is no practical way to obtain accelerated healing in an otherwise healthy individual. In the future there may, however, be a practical application in those patients who for some reason, such as malnutrition, demonstrate a delay in wound healing.

ANTIBIOTICS AND ABSORPTION

Several groups of investigators have reported that the administration of antibiotics to chicks results in a reduction in the thickness of the intestinal wall (M. E. Coates, *et al., Chemistry and Industry 1333 (1953)*; W. F. Pepper *et al., Poultry Sci.* **32,** *656 (1953)*; H. J. Jukes *et al., Ibid.* **35,** *716 (1956)*). It has never been established clearly, however, whether the thickening in the absence of antibiotics, which may be a reaction to the presence of pathogenic organisms in the lumen of the gut, is associated with any specific change in physiological activity.

Experiments on animals have indicated that utilization of protein is made more efficient by the administration of antibiotics (T. C. Huang and C. M. McKay, *J. Nutrition* **50,** *129 (1953)*; R. M. Forbes, *Ibid.* **53,** *275 (1954)*), but the methods employed failed to distinguish between differences in the degree of protein hydrolysis in the gastrointestinal tract and differences in absorption of the end products. B. B. Migicovsky *et al.* (*Arch. Biochem. Biophys.* **34,** *479 (1951)*) observed an increase in uptake of Ca^{45} by penicillin-treated chicks, and R. Burgess *et al.* (*Ibid.* **33,** *339 (1951)*) noted an enhanced absorption of vitamin A when antibiotic was employed.

In order to test this notion of better absorption by thinner walled digestive organs, H. H. Draper (*J. Nutrition* **64,** *33 (1958)*) has compared the absorption of a standard oral dose of radiolysine in penicillin-treated and control chicks. The use of a specific amino acid with a radiolabel circumvented any possible effect of the antibiotic on protein digestion, and, at the same time, provided a sensitive means of detecting small differences.

In a first experiment, nine female chicks (34 days old) were selected at random from each of two groups of 16 birds which had been maintained since hatching on a practical basic diet including vitamins A, D, riboflavin, calcium pantothenate, menadione, nicotinic acid, choline and cyanocobalamin. Group one was maintained as a control; group two received, in addition, 100 mg. of procaine penicillin per kg. of basal diet.

Each selected chick was given an oral dose of 0.39 mg. of uniformly labeled C^{14}-L-lysine. The birds were sacrificed 150 minutes later, during which time access to the basal diet was permitted. The small intestines and ceca were removed, incised longitudinally, and the contents were flushed out. The lengths of the relaxed small intestines were measured in the horizontal position and the weights of the intestines and ceca were recorded after blotting to remove excess moisture.

Counting of the intestinal and cecal contents revealed that approximately 90 per cent of the dose of radiolysine had disap-

peared from the digestive tract after two and one-half hours. There was 15 to 41 per cent lower activity of the intestinal and cecal contents in the penicillin-treated chicks as compared to non-penicillin-treated control animals. In conformity with the findings of others, the weights of the small intestine and ceca were significantly less in the group that had received penicillin. No significant changes were observed in the lengths of the small intestines.

According to the author, these findings imply that penicillin caused a reduction in the weight of the chief organs of digestion and absorption, thus increasing the uptake of free lysine, but the conclusion that the second result was a direct consequence of the first is subject to certain qualifications. The occurrence of a shift in bacterial populations in the gut under the influence of penicillin, with a consequent loss of the isotope as $C^{14}O_2$ arising from fermentation, or an acceleration of food passage, could also account for the results.

Since several workers (J. P. Hillerman, *et al.*, *Poultry Sci.* **32,** *332* (*1953*); E. L. Stokstad, *et al.*, *Ibid.* **32,** *1054* (*1953*); and H. G. Jukes, *et al.*, *Ibid.* **35,** *232* (*1956*)) have reported an increase in rate of food passage through the digestive tract of birds under the influence of antibiotics, a second experiment was carried out in which the chicks were sacrificed before sufficient time had elapsed for the radiolysine dose to be excreted in the feces.

In this case the penicillin treatment had failed to alter the weights of the small intestines and ceca as compared to the control chicks. No differences could be detected with respect either to rates of passage or rate of absorption. Thus, the author concludes that the original premise holds true (*i.e.* that absorption was more efficient from the lighter intestinal tract of the penicillin-fed birds than from the heavier tract of the control chicks).

These studies should be repeated with other substrates and in other species, before the phenomenon can be regarded as general.

THE TRANSPORT OF NON-ESTERIFIED FATTY ACIDS IN BLOOD

Largely deriving from the laboratories of V. P. Dole and R. S. Gordon, Jr., there has been a large body of experimental results establishing the importance of the non-esterified fatty acids (NEFA) of blood plasma as an important vehicle of transport of fatty acids from adipose depots to other tissues for oxidation when other energy sources are not readily available. Certain aspects of this important topic have been reviewed previously (*Nutrition Reviews* **16,** *103* (*1958*); **16,** *135* (*1958*)).

Considerable information is available concerning the physical state of the NEFA in the plasma. It is incorrect to consider these substances as "free" fatty acids because in the circulation they are nearly entirely associated with proteins. The predominant role of albumin in the binding of non-esterified fatty acids was suggested by the difficulty experienced by physical chemists in preparing albumin free of fatty acids. This association proved to have practical significance in that it was observed that the presence of fatty acids stabilized solutions of purified albumin.

The nature of albumin binding of fatty acids has been the subject of systematic study by D. S. Goodman (*J. Am. Chem. Soc.* **80,** *3892* (*1958*)). Human serum albumin which had been freed as completely as possible from fatty acids was equilibrated with fatty acid in an aqueous buffer in the presence of n-heptane. The technique, which is known as partition analysis, permits the accurate measurement of the small concentration of fatty acid which is in free solution, as contrasted to the much larger

amount of fatty acid which is bound to the albumin. The measurement of the degree of binding which occurred at increasing concentrations of unbound fatty acid provided information concerning the number and the affinity of the binding sites on albumin.

The results indicated that there were two binding sites of the highest affinity with association constants for stearic acid of 8.0×10^7. This degree of affinity of albumin for fatty acids exceeds the binding by albumin of most other substances which have been studied. Five sites on the albumin molecule were available to bind fatty acids with lesser affinity. There were at least 20 additional sites of even lower affinity for fatty acids molecules.

The binding sites of the highest affinity appeared to be mutually independent. The affinity of binding of different fatty acids, when measured, provided some interesting information. The binding of the prevalent saturated fatty acids of 16 and 18 carbon atoms was much greater than that of shorter fatty acids. The addition of one double bond in the fatty acid molecule as in oleic acid increased the binding by albumin 40 per cent, whereas the addition of a second double bond reduced the binding of linoleate as compared to oleate.

It is important to consider the above measurements in terms of the physiologic transport of fatty acids. In the body the concentration of unesterified fatty acids is about 0.5 mEq per liter or 5×10^{-4} M. At this concentration the number of molecules of fatty acid bound per molecule of albumin is less than one. Under most circumstances, therefore, the binding of fatty acids by the binding sites of highest affinity would not be saturated, and the concentration of fatty acids in free solution would be less than 0.01 per cent that which is bound.

Some information is available concerning the degree that other blood components can bind non-esterified fatty acids. E. Shafrir (*J. Clin. Invest.* **37,** *1775 (1958)*), has measured the concentration of non-esterified fatty acids in albumin separated from serum by 60 per cent saturated ammonium sulfate. Only about 77 per cent of the total serum non-esterified fatty acids were found in this fraction. The albumin in the serum of patients with nephrosis was found to contain a fraction of the total non-esterified fatty acids which was even smaller. These observations were supported by measurements of non-esterified fatty acid content of lipoproteins which were separated by ultracentrifugation.

These results would indicate that a considerable fraction of the total serum unesterified fatty acid is in combination with lipoprotein. In contrast to these findings, the authors refer to unpublished experiments conducted with D. S. Goodman in which the binding of lauric, palmitic, stearic, oleic and linoleic acids by lipoproteins were measured by the addition of the above fatty acids labeled with carbon-14. On the basis of these observations, it was estimated that less than 1 per cent of the serum non-esterified fatty acids would be bound by low density lipoproteins. The reason for these conflicting estimates of the degree of binding of non-esterified fatty acids remains to be determined. The suggestion was made that the fatty acids isolated with lipoproteins by physical methods might differ chemically from the fatty acids added *in vitro*.

It is very difficult to reconcile the extraordinary biologic activity of the non-esterified fatty acids of plasma with the high degree of protein binding which has been described. The half-life of the plasma non-esterified fatty acids is two to three minutes in the fasting subject. To achieve this high rate of transfer to the cells, binding sites of the cells with affinities approaching that of the serum proteins might be anticipated. A beginning has been made in the study of tissue binding of non-esterified fatty acids by D. Goodman (*J. Clin. Invest.* **37,** *1729 (1958)*), who has measured the distribution of added labeled fatty acid between erythrocytes, erythrocyte capsules ("ghosts") and

albumin solution. Analysis of the results indicated that there were 3.2×10^7 binding sites for palmitate on each erythrocyte and the association constant for each of these sites was 2.2×10^6.

This apparent association constant of binding by red blood cells is more than an order of magnitude less than the binding sites of albumin which bind fatty acids at physiologic concentrations of NEFA. Under normal conditions, therefore, erythrocytes carry less than 1 per cent of the NEFA. The affinity of the ghosts for palmitate was essentially that of the intact red cell, supporting the view that the binding sites existed on the cell membrane. It was shown further that the affinity of the binding sites was not dependent on metabolic activity.

Examination of NEFA binding by more representative tissue cells presents many technical problems. D. L. Fillerup, J. C. Migliore, and J. F. Mead (*J. Biol. Chem.* **233,** *98 (1958)*) have measured the uptake of palmitate-1-C^{14} by ascites tumor cells. When respiration was blocked by cyanide a large fraction of the labeled palmitate was found bound by the cells. This led the authors to suggest that the fatty acid may have combined with components of the cell surface.

Despite the fact that a transfer of fatty acid has apparently been demonstrated between albumin solutions and red blood cells and ascites tumor cells, a direct transfer probably does not occur. The incredibly fast disappearance of non-esterified fatty acids from the blood indicates a flux out of the vascular compartment considerably faster than the outward flux of albumin.

The possibility must be considered that the extremely small concentration of fatty acid in simple aqueous solution is responsible for the rapid exit from the blood. The role of albumin and other binding agents of the blood may not be one of transport to the cells but one of stabilizing the level of the diffusible fatty acid at a low level.

In summary: The non-esterified fatty acids which are of such great importance in fatty acid transport, circulate largely bound to serum albumin. Two binding sites on the albumin molecule with high affinity for NEFA are not saturated with fatty acids during the physiologic fluctuations of fatty acid concentration. Some fatty acids, the nature of which remain to be established, may be associated with lipoproteins.

Erythrocytes bind less than one per cent of the non-esterified fatty acids of blood. Binding sites for fatty acids have been demonstrated on the surface of red cells and probably exist on other cells. The fatty acids which are free in a physico-chemical sense may be responsible for the extremely rapid outward flux of fatty acids from the circulation.

SUPPLEMENTATION OF FLOUR

Although wheat flour contains considerable protein (13 per cent of the total calories) less than one-half of this protein is available to the animal for the formation of new tissue (R. J. Block and H. H. Mitchell, *Nutr. Abs. & Rev.* **16,** *249 (1946–47)*). Protein inadequacy of wheat flour is generally considered to be due to insufficient lysine.

As early as 1917, T. B. Osborne and L. B. Mendel (*J. Biol. Chem.* **32,** *369 (1917)*) suggested that small additions of the more efficient proteins can supplement an inadequate cereal protein. Obviously, for the greatest economy, an attempt should be made to achieve a combination of proteins and amino acids which will be as complete as possible in essential amino acids with a minimum percentage of waste protein.

H. W. Howard, W. J. Monson, C. D. Bajer, and R. J. Block (*J. Nutrition* **64,** *151 (1958)*) have focused attention on this problem of protein enrichment with special

reference to bread flours. From an extensive review of the literature, they too were led to the conclusion that what is desired is a protein mixture which will supply the optimal proportions of amino acids.

Although the composition of this ideally balanced "complete protein" is not actually known, evidence available on the amino acid composition of various adequate and inadequate diets, of animal tissues, of food proteins (especially whole egg) and of the minimal amino acid requirements of many species of animals, has caused this group to assume tentatively that a "complete protein" should contain 5.3 g. of lysine per 16.0 g. of protein nitrogen.

Thus, the supplemental value of a food protein, or another food in which the limiting amino acid is lysine, can be calculated by the equation $S = \frac{QL}{5.3}$ where: S is the supplemental value per 100 g; Q is the percentage of crude protein ($N \times 6.25$) in the supplement; L the grams of lysine per 16 g. nitrogen in the supplemental protein food.

The supplementary value of any combination of two food proteins may be calculated from the equation $S = S_1(X) + S_2(1 - X)$ where: S = the supplementary value of the total product; S_1 = the supplementary value of the first protein; S_2 = the supplementary value of the second protein; X = the fraction of S_1 in the mixture.

Actual calculations indicate that on a weight basis, lysine hydrochloride is the supplement of choice within the limitations described above. Non-fat dry milk solids have limited supplemental value since the protein concentration is low. Isolated soybean proteins provide a concentrated source of "complete protein" but supply so little extra lysine that their supplementary value is rather limited. Lactalbumin has a very high supplementary value because, in addition to the added "complete protein" furnished, it also provided considerable extra lysine. While the addition of wheat gluten to bread increases the total protein it does not improve the quality of the protein.

Several experiments were carried out to test the validity of the above calculations. A preliminary feeding trial with weanling rats established that the protein in a high-protein flour and that in a standard low-protein (72 per cent extraction flour) were equal in nutritive value when fed at equal levels of nitrogen. This was demonstrated by dilution of the high-protein flour with wheat starch.

The nutritional effects of supplementing wheat flour with lactalbumin, non-fat dry milk solids, soybean proteins, wheat gluten and lysine were investigated. For these studies 144 weanling male rats weighing 45 g. to 50 g. each were distributed randomly among 18 groups of eight rats each. After 28 days on the experimental diets, all animals were sacrificed. The entire gastrointestinal tract and contents were removed and the carcasses were frozen. The carcasses of all animals from each dietary group were then pulverized and thoroughly mixed. Aliquots from each pooled sample were analyzed in triplicate for moisture, fat and protein.

Initial and final weights were noted, as were weight gains for each group along with the quantities of crude protein and "complete protein" consumed and the average daily gain in per cent. The correlation between growth rate and calculated "complete protein" was found to be much better than that between growth rate and crude protein. This high degree of correlation between growth rate and dietary level of "complete protein" confirms the idea that lysine is the primary limiting amino acid in white flour alone and in combination with practical levels of supplementary proteins.

It is of interest to note that food intake and protein content of the carcasses were also closely correlated with the dietary level of crude protein. P. D. Deshpande *et al.* (*J. Biol. Chem.* **230,** *327, 335 (1958)*) have

also found that food intake is reduced on diets containing a deficiency of one of the essential amino acids. Thus it is possible to predict with remarkable precision, from the dietary level of "complete protein," the growth rate, the voluntary food intake and the protein content of the carcass.

The experimenters are of the opinion that, under conditions in which some other nutrient (calories, vitamins, minerals, etc.) may be limiting, it should be possible to show a similar relationship by which the growth rate, voluntary food intake and body composition could be predicted from the dietary "level of completeness". By "level of completeness" is meant the nutrient content expressed as a fraction of the optimum requirement.

NATURE OF THIAMINE-SPARING BY DIETARY ANTIBIOTIC

Oral administration of antibiotics has been reported to counteract in part the effects of a limited dietary intake of thiamine and certain other B-vitamins (*Nutrition Reviews* **10,** *107, 294 (1952)*; **14,** *124 (1956)*). Several explanations have been advanced as to the mechanism involved in vitamin-sparing by antibiotics. The phenomenon of vitamin synthesis, particularly thiamine, by microorganisms in the intestinal tract seems fairly well established. An increase in absorption of the vitamin, brought about either directly or indirectly by the antibiotic, offers another possibility as to mechanism of action. This is supported by the recent observation that C^{14}-labelled L-lysine is absorbed more readily from the gut of chicks receiving dietary penicillin (H. H. Draper, *J. Nutrition* **64,** *33 (1958)*). A direct systemic effect of antibiotic on the host animal, resulting in a smaller net requirement for certain nutrients, must also be considered as a possible mode of action.

The use of germ-free animals in studies of this nature should serve to clarify the direct role, if any, that antibiotics might have in nutrition. Within the framework of interest of the Lobund Institute, University of Notre Dame, Notre Dame, Indiana, regarding the role of microflora in animal nutrition, studies have been conducted to assess the role of penicillin in the nutrition of both germ-free and normal stock rats (B. S. Wostmann, P. L. Knight and J. A. Reyniers, *J. Nutrition* **66,** *577 (1958)*).

Germ-free weanling rats, normally three males and three females to a group, were divided into three groups. Group one was fed a complete semi-synthetic diet; group two was fed the same diet with thiamine omitted; and group three received the thiamine-low diet plus 50 mg. per kilogram of radiation-sterilized procaine penicillin G. The thiamine content of the three diets, by analysis, was found to be 9.4, 0.4, and 0.4 milligrams per kilogram for groups one, two, and three, respectively. This experimental design was duplicated with normal stock animals.

The results reported were from three experiments, with the exception that the group receiving the complete diet without penicillin was not included in the first experiment. After 28 days, the germ-free animals on the thiamine-deficient diet were in poor condition and those not sacrificed died between the fourth and fifth week. The normal stock rats on the same diet were in better physical condition, and survival extended to the tenth week in animals that were not sacrificed. In both the germ-free and the normal stock animals, the stomach contents indicated extensive coprophagia, in spite of the fact that the rats were housed on raised screen floors.

The data confirmed the view that the antibiotic was of no benefit in germ-free animals that were restricted in their thiamine intake. The authors noted further that

for all experimental groups, the normal stock animals had a more healthy, livelier appearance and were generally in better condition than their germ-free counterparts.

A comparison of the thiamine content of the livers, in animals sacrificed after the 28-day experimental period, revealed that thiamine storage in germ-free animals fed the complete diet was considerably below normal (3.74 micrograms per gram of fresh liver). The livers of normal stock animals on the same complete diet contained 8.57 micrograms per gram. On the thiamine-deficient diet, liver depletion of thiamine stores was evident (0.38 and 0.50 micrograms per gram for germ-free and normal stock animals, respectively).

The liver storage data supported the view that penicillin was beneficial to the normal stock animals but not to the germ-free animals. Liver thiamine levels were 0.47 and 1.16 micrograms per gram for the antibiotic-supplemented germ-free and normal stock animals, respectively.

The body store of thiamine, as measured by liver storage, was even more strikingly different in view of the influence of the antibiotic on liver weight. For example, the livers of thiamine-deficient normal stock animals (penicillin-supplemented) averaged 4.21 g. per 100 g. of body weight, while the livers of comparable germ-free animals averaged only 2.92 g. per 100 g. of body weight. Thus the total thiamine in the livers of the normal stock animals receiving the thiamine-deficient diet plus penicillin was nearly four times as high as in the comparable germ-free animals.

From these results the authors conclude that the failure of penicillin to counteract the detrimental effects of a thiamine-low diet in germ-free rats is a strong indication that the beneficial effects of the antibiotic in a contaminated environment is mediated through an action on the intestinal flora.

Whether or not the extra thiamine made available to the animal through intestinal microbial synthesis was absorbed during "first passage," or whether it represents absorption after re-cycling as a result of coprophagia, is an important question that remains unanswered. In this connection the suggestion by Draper (*loc. cit.*) that oral antibiotic effects a reduction in the weight of the intestinal tract tissues, which in turn might influence the efficiency of nutrient absorption, has been supported by studies in the chick (H. A. Gordon, M. Wagner, and B. S. Wostmann, *Antibiotics Annual* **248**, (*1957–58*)).

The observation that normal animals fed the complete diet appeared to be more "normal" than germ-free animals fed the complete diet raises some doubt as to whether the animals used in the Notre Dame experiments were actually comparable as weanlings or at the initiation of the experiment. Data relative to the liver stores of thiamine in germ-free weanling and normal stock animals would, for example, have been most helpful in evaluating more critically the influence of the experimental treatments on the thiamine status of the animals.

WATER-SOLUBLE VITAMIN K ANALOGUES

There is a valid rational basis for the development and use of water-soluble vitamin K analogues. For instance, the vitamin K deficiency resulting from poor absorption of fat-soluble substances would not be expected to respond to oral doses of the fat-soluble K_1 or K_2. Moreover, intravenous administration of a water-soluble form of the vitamin might be more convenient and bring more rapid response than would that of an emulsion.

However, in practice, water-soluble analogues of menadione (2-methyl-1,4-naphthoquinone) such as menadiol sodium diphosphate (sodium 2-methyl-1,4-naphthohydroquinone - 1,4 - diphosphate) are

relatively ineffective in correcting the hypoprothrombinemia induced by oral anticoagulants (D. F. James, *et al.*, *A.M.A. Arch. Int. Med.* **83,** *632 (1949)*). Moreover, the water-soluble menadione derivatives may show some toxic symptoms in infants (*Nutrition Reviews* **15,** *331 (1957)*).

For these reasons, C. W. Mushett, K. L. Kelley and R. Hirschmann (*Blood* **14,** *37 (1959)*) investigated the physiological properties of water-soluble derivatives of vitamin K_1, especially disodium 2-methyl-3-phytyl-1,4-naphthohydroquinone-1, 4-diphosphate, with special reference to the counteraction of drug-induced hypoprothrombinemia.

The investigation was carried out using beagle dogs with groups of three experimental animals and two or three control dogs for each test. For the curative test the anticoagulant, dicumarol or dipaxin (2-diphenylacetyl-1,3-indanedione), was given orally on two successive days followed, after two or three days, by the vitamin K analogue. Prothrombin times were taken thereafter at one, two and one-half and five hours. For the prophylactic test, both the anticoagulant and vitamin K analogue were given daily for four days and prothrombin times were then followed for 11 days.

When the doses of dicumarol were 6 and 2 mg/kg, both K_1 emulsion and dihydrovitamin K_1 diphosphate, given intravenously at 2 mg/kg (expressed in terms of K_1), restored prothrombin time to normal in about five hours. However, when the dicumarol was given in two doses of 4 mg/kg, the prothrombin time of the dogs given the water-soluble analogue again rose in one or two days. The same results were obtained following oral administration of both substances at 5, 10 and 20 mg/kg. However, some rebounds were observed for both substances at the lowest level and in one case for the diphosphate at the intermediate dose. In both cases it appeared that the water-soluble compound acted somewhat more rapidly but that the duration of its action was shorter.

Following intramuscular administration of the diphosphate (5 or 10 mg/kg.) normal prothrombin time was re-established within 24 hours, whereas that of the K_1-treated animals was normal only after four days. Examination revealed that the intramuscularly injected emulsion had broken down, leaving droplets of oil to be absorbed slowly. In a test of the production of a refractory state by the vitamin K analogues, it was found that after K_1, neither dicumarol nor dipaxin produced a very marked rise in prothrombin time, whereas dihydrovitamin K_1 diphosphate had no effect of this sort.

In the prophylactic tests, vitamin K_1 emulsion given intravenously was considerably more effective than the diphosphate.

From these experiments it can be concluded that disodium 2-methyl-3-phytyl-1,4-naphthohydroquinone-1,4-diphosphate (and also the monophosphate) are as effective as vitamin K_1 in the reversal of dicumarol - induced hypoprothrombinemia when given orally or intravenously, and considerably more so when administered intramuscularly. The effect of the water-soluble compounds is established more rapidly but for a much shorter duration. This knowledge has led the authors to propose several cases in which the use of the water-soluble compounds would be advantageous. For example, in cases in which neither oral nor intravenous administration is feasible, as in infants and the aged, the intramuscular route might be preferable using the diphosphate. Further, in cases in which anticoagulant therapy must be interrupted by surgery and then immediately resumed, the use of a vitamin K analogue with a short duration of effectiveness seems indicated.

These studies have also opened the way for more fundamental experiments on vitamin K action at the cellular level. In the study of the action of vitamin K derivatives in oxidative phosphorylation (*Nutrition Reviews* **14,** *211 (1956)*), the availability of a water-soluble vitamin K should facilitate

enzyme studies. Moreover, it will enable a true comparison to be made of water-soluble derivatives of vitamin K_1 and those of menadione, which does not possess the phytyl group and has a very different action in mitochrondrial preparations.

It will also be important to identify the true active form of this vitamin. At present it is uncertain whether the phytyl side chain of vitamin K_1 is retained during its action or whether a phytyl or some other chain is added to the menadione nucleus.

VITAMIN E IN THE CHICK

Symptoms of vitamin E deficiency (exudative diathesis) in the chick can be produced in approximately three weeks by the use of a diet containing torula yeast as the protein source (M. L. Scott, *et al.*, *J. Nutrition* **56,** *387 (1955)*). These symptoms are prevented with either 5 mg. α-tocopheryl acetate per pound of diet, or 10 per cent dried brewers' yeast. Addition of a variety of antioxidants has failed to alleviate the deficiency. J. Goldstein and M. L. Scott (*J. Nutrition* **60,** *349 (1956)*) have found that the deficiency results in a decrease in total plasma protein, particularly albumin. Since the exudates appearing in exudative diathesis are found to be similar to the plasma as determined electrophoretically, a possible role of vitamin E in capillary permeability is suggested.

An extension of the above studies on the possible mechanisms of vitamin E deficiency in decreasing albumin in the serum has been carried out by B. G. Creech, M. M. Rahman, B. L. Reid and J. R. Couch (*J. Nutrition* **64,** *65 (1958)*). The production of exudative diathesis was accomplished by the use of a torula yeast diet.

The basal diet was identical to that used in previous studies (B. G. Creech *et al.*, *J. Nutrition* **62,** *83 (1957)*) except that it was adjusted to the nutrient requirements of chicks and cerelose was substituted for lard in experiments where the effect of lard was to be determined. The ration was calculated to contain 22 per cent of protein and was considered to be adequate in all known nutrients except vitamin E. When dried brewers' yeast was added to the diet, the supplementation was made on an equi-protein basis for torula yeast.

Because other symptoms of vitamin E deficiency, such as encephalomalacia, have been caused by inclusion in the diet of dietary stress factors such as unsaturated fats and fish oils, it was thought necessary to observe the effect of such supplements in the production of exudative diathesis. The production of this symptom was not accelerated by the inclusion of dietary lard. Moreover, since supplementation of the diet with 20 mg/lb of α-tocopheryl acetate or 10 per cent of dried brewers' yeast completely prevented the development of the condition in the presence or absence of lard in the diet, it appears that exudative diathesis does not arise primarily from the action of vitamin E as an antioxidant. The addition of 0.025 per cent of butylated hydroxy toluene, both in the presence and absence of added fat, was also without effect in preventing the occurrence of the disorder.

Although exudative diathesis appears not to be associated with the anti-oxidant functions of vitamin E, determination of the rancidity index (reciprocal of iodine number) of feed stored for one week was found to shed more light on this aspect of the problem, as well as on the function of brewers' yeast in the prevention of this condition. Very little oxidation had taken place in the lard prior to the time of mixing in the diets. After a period of a week, the rancidity index had increased, but not to an excessively high level. Vitamin E and the antioxidant butylated hydroxy toluene effectively retarded oxidative rancidity of the fat when added to

the diet, but dried brewers' yeast was totally ineffective as an antioxidant.

Total protein values were somewhat higher in the groups receiving vitamin E, but these differences were not found to be significant. In the deficient birds a highly significant reduction in albumin was noted which could be increased by the addition of vitamin E. Decreases in albumin were accompanied by increases in globulin which, the investigators feel, were attempts by the body to counteract the effect of the albumin reduction.

In most cases, examination of animals showed a thick gelatinous mass covering the breast just under the skin. However, in some cases fluid exudates were obtained in sufficient quantities to allow chemical and electrophoretic investigation. The exudates were found to contain a relatively higher proportion of albumin than does the serum in vitamin E-deficient birds. Otherwise, the exudate and serum were quite similar in composition. The reduction of albumin in the serum was not completely accounted for by the higher level in the exudate, and the authors are of the opinion that it is possible that albumin synthesis may be affected in vitamin E deficiency even though the primary factor in appearance of larger amounts of albumin in exudates is probably that of increased capillary permeability.

To test further the possibility of the involvement of vitamin E in albumin synthesis, an experiment was set up involving analysis of blood samples at close intervals during the development of exudative diathesis. Blood hemoglobin had been seen previously to be a reliable index of the anemia accompanying vitamin E deficiency; therefore determination of this factor was carried out on each blood sample to ascertain if the anemia is progressive or a result of hemorrhaging at the time of appearance of exudative diathesis.

Until the seventh day on the diets, no differences could be detected in any of the protein components or in blood hemoglobin. Between the seventh and tenth days, slight differences in total protein appeared which became significant by the eighteenth day. The reduction in total protein was found to be due to a reduction in albumin in the deficient birds. During the course of the experiment the globulin level increased with time, but only very small differences were seen between groups. No difference was noted in hemoglobin values until the eighteenth day, when symptoms of exudative diathesis first appeared. A significant lowering of the hemoglobin values at this time in the vitamin E deficient group suggested to the authors that the anemia was due primarily to hemorrhaging.

Individual birds required varying lengths of time to develop symptoms of exudative diathesis. The authors are of the opinion that this was probably due to an inherent difference in ability of hens to deposit vitamin E in the egg, resulting in differing levels of the vitamin stored for use by the chick. It was felt that this might affect the results of experiments when random selection of birds for analysis was employed. Accordingly, the birds were re-examined and classified as severely affected and slightly affected. Significant differences in total protein, albumin and hemoglobin concentration were detected between the severely and slightly affected birds.

The large difference in albumin could be due either to impaired synthesis or to the fact that albumins are the first protein molecules to escape the blood with increased capillary permeability. Comparison of serum and exudate from severely affected and slightly affected birds indicated further the possibility of loss of the smaller albumin molecules to a greater degree than the other serum proteins. In both severely and slightly affected birds the albumin concentration of the exudate was higher than in the serum.

It seems probable that this experiment could be carried out in a much more definitive manner with the employment of radio-

isotopes. Intravenous injection with S^{35}-labeled amino acid and subsequent time-sequence analysis of the radioactivity of components of serums and exudate should do much to elucidate the problem of a deficiency of vitamin E interfering with albumin synthesis as compared to albumin loss through the capillary walls.

NUTRITION IN HYPERTHYROIDISM

Liver preparations have been reported to reverse growth retardation almost completely and to prevent the high mortality rates observed in hyperthyroid rats (B. H. Erschoff, *Arch. Biochem.* **15,** *365 (1947)*). Levels of known vitamins above those usually fed in purified casein-sucrose diets do not improve growth significantly in normal animals, but quantitative requirements for certain of the B-complex vitamins are elevated in the hyperthyroid rat.

J. J. Betheil and H. A. Lardy (*J. Nutrition* **37,** *495 (1949)*) observed that crystalline vitamin B_{12}, or highly purified liver extracts rich in this vitamin, promoted growth in the hyperthyroid rat. However, dried whole liver preparations promoted growth to an extent that could not be accounted for by their vitamin B_{12} content. Moreover, a treatment which should have removed vitamin B_{12} was not successful in removing the growth factor(s) present.

C. C. Stevens and L. M. Henderson (*J. Nutrition* **64,** *67 (1958)*) have undertaken the search for possible additional unidentified growth factors for the hyperthyroid rat. These workers were able to facilitate evaluation of liver fractions by the use of a three-week "curative" assay which gave more reproducible results than the five-week "preventive" assay commonly employed in such studies. Basal rations were of two main types. The corn oil basal ration included per kilo: sucrose, 68.8 per cent; casein, 22 per cent; corn oil, 1 per cent; a salt mixture patterned after the ash of milk, 4 per cent; L-cystine, 2 g; and 4 per cent of a sucrose-vitamin mixture containing 30 mg. thiamine, 30 mg. riboflavin, 50 mg. niacin, 50 mg. calcium panothenate, 30 mg. pyridoxine, 5 mg. folacin, 0.2 mg. biotin, 200 mg. p-aminobenzoic acid, 15 mg. vitamin B_{12} and 1 g. choline. The fat-soluble vitamins were given as a commercial preparation containing vitamins A and D. The hydrogenated vegetable oil basal diet was approximately the same with the exception that 12 g. of protamone (3.07 per cent thyroxine in one part iodinated casein and four parts sucrose) and 11 g. of crisco were added.

All rats were first fed the corn oil basal ration for one week during which time the average weight gain was approximately 30 g. Growth studies were carried out along two experimental designs, the preventive assay and the curative assay. For preventive assays, adapted rats were divided into two groups, one (negative control) receiving the hydrogenated vegetable oil basal ration, the other (positive control) receiving the same diet except that protamone was omitted. Materials under study were added to the hydrogenated vegetable oil basal rations, and these supplemented diets were fed to other groups of animals. The curative assays employed rats which had been depleted for two weeks of the substance in question following the adaption period. During this depletion, the animals all received the hydrogenated vegetable oil basal rations (with and without protamone) employed in the preventive assay.

Ten per cent pork liver residue was added to the hydrogenated vegetable oil basal rations at the expense of an equal quantity of casein and sucrose. In the curative assay the supplement was, of course, administered after a depletion period.

Under preventive assay conditions the first reliable indication of growth promotion by ten per cent pork liver residue was given by growth data at the end of four weeks. In

contrast, the curative assay consistently showed growth promotion to the extent of 60 ± 10 per cent within two weeks of the addition of 10 per cent pork liver residue to the ration.

Many naturally occurring materials were tested for growth promoting activity in the thyrotoxic rat under curative assay conditions. Those included: 5 per cent pork liver residue, 10 per cent liver residue, 2.7 per cent lipid fraction of pork liver residue, 3.65 per cent defatted pork liver residue, 7.3 per cent defatted pork liver residue, 2.7 per cent lipid fraction and 7.3 per cent defatted pork liver residue, 0.27 per cent lipid fraction and 7.3 per cent defatted pork liver residue, 10 per cent beef-liver residue, 10 per cent wheat germ oil, 3 per cent lipids from whole pork liver, 10 per cent soybean protein, 10 per cent dried brewer's yeast, 25 per cent dried brewers' yeast, and 11.8 per cent casein.

Of all the materials tested, water-extracted pork liver residues were consistently the most active. Casein and the other proteins were inactive. Water-extracted beef liver residues were significantly less active than the corresponding pork liver residues. Both the lipid extract and defatted residue were found to have growth-promoting activity. Recombinations of these fractions supported growth as effectively as the original pork liver residue, in fact the best growth response was obtained with a combination of these two fractions in proportions different from those present in the original liver preparation.

Since activity was consistently retained in the lipid portion, further study of this fraction was undertaken by the method of addition of known nutrients. Cholesterol alone had no appreciable growth-promoting activity, but it had a small effect when fed with 5.5 per cent corn oil. Corn oil alone was relatively ineffective as a growth stimulus. Increasing the total vitamin level five-fold, or lowering it ten-fold had no effect on growth but very significant growth stimulation was consistently obtained with crystalline aureomycin.

A combination of cholesterol, corn oil and aureomycin elicited growth responses approximately equal to those given by 10 per cent pork liver residue alone, but supplementing the basal diet with a combination of all four of these materials gave a growth rate approaching that observed when iodinated casein was removed from the ration. The marked growth response observed under these conditions supports the hypothesis that pork liver residue contains growth factor(s) distinct from cholesterol or fatty acids.

The possibility of liver-contained factors directly antagonistic to the metabolic action of the thyroid hormone was tested by measuring basal metabolic rates. No significant difference was observed in these rates upon feeding 10 per cent liver.

A major difference between these results and others in the literature was the lack of response to additional casein either with or without added cholesterol and corn oil. Several other supplements, which are composed primarily of protein, promoted growth only slightly.

It is not possible from these results to decide whether the unidentified growth factor acts as a hormone, a vitamin, or a detoxifying agent.

SOFT TISSUE CALCIFICATION

The occurrence of soft tissue calcification and stiff wrist joints in guinea pigs on certain diets has been reported by at least two groups of investigators (R. Wulzen and A. M. Bahrs, *Am. J. Physiol.* **133,** *500 (1941)*; W. J. van Wagtendonk and R. Wulzen, *Arch. Biochem.* **1,** *373 (1943)*; S. E. Smith *et al. J. Nutrition* **38,** *87 (1949)*). The evidence of Smith *et al.* suggested, however, that the wrist stiffness and tissue

calcification were not part of the same syndrome.

In continuing studies, therefore, they concentrated attention on soft tissue calcification which seemed to be the more significant abnormality. Various purified diets were employed, all of which produced some degree of calcification in one or more organs and tissues as demonstrated by chemical analysis, histological study or X-ray, as opposed to the absence of calcification in animals fed a stock diet. Growth was generally poor on the purified diets; there was considerable mortality and a more suitable basal purified diet was recognized as a definite need. Such a diet was developed by M. E. Reid and G. M. Briggs (*J. Nutrition* **51,** *341* (*1953*)) and was found to produce satisfactory growth and viability and no calcification of soft tissues.

It was postulated that the calcification observed on other purified diets might be the result of inappropriate magnesium-calcium-phosphorus relationships in the diet (*Nutrition Reviews* **2,** *189* (*1944*); **15,** *232* (*1957*); **16,** *173* (*1958*)). L. A. Maynard, D. Boggs, G. Fisk and D. Segwin (*J. Nutrition* **64,** *85* (*1958*)) have now carried out a series of studies aimed toward the testing of this hypothesis.

Young rats were weaned at one week of age and divided into three groups. Two of these groups were started on a stock diet stated by the manufacturers to contain ground whole wheat, ground whole oats, alfalfa leaf meal, soybean oil meal, soybean oil, irradiated brewer's yeast, bone meal, limestone, iodized salt and additional sources of various vitamins. This diet was guaranteed to contain 18 per cent protein. The Reid-Briggs diet consists of casei (30 per cent), corn oil, sucrose, cellulose spangles, starch, cerelose, a salt mixture and a supplement containing 15 vitamins. Shortly after weaning, the animals were subdivided into groups of seven animals each and were placed on experimental diets varying in their content of Mg, Ca and P as follows: (1) Stock (2) Reid-Briggs (3) Reid-Briggs low Mg (4) Reid-Briggs low Mg, Ca, P (5) Reid-Briggs low Mg, Ca (6) Reid-Briggs low Mg, P. The experimental periods varied from 13 to 15 weeks.

When the animals were slaughtered, the serum was saved for analysis and, following a gross post-mortem examination, tissue samples were taken for chemical analysis and histological examination. Growth was found to be satisfactory on both the stock and Reid-Briggs diets, being 4.4 g. and 4.3 g. per day, respectively. All animals were in good condition. By contrast, the animals on the Reid-Briggs diet in which the magnesium content had been sharply reduced made an average daily gain of only 2.2 g., and there was a marked loss of hair, lessened activity and poor muscular coordination of the rear legs.

Gross post-mortem examinations revealed no abnormalities in the animals fed on a stock or complete Reid-Briggs diets. In contrast, on diets low in magnesium, the kidneys were enlarged, white in color and were about double the weight and volume of those of animals of comparable size on other diets. The white spots were found to represent focal necrosis, which in the case of animals on low-magnesium diet was accompanied by calcification. Hearts and muscles appeared to be normal. The calcium levels of the serum were normal on all diets.

This was also true for magnesium in the case of the stock and Reid-Briggs diets, but when the magnesium content of the latter was lowered, its level in the serum was reduced by approximately 40 per cent. When the magnesium was lowered, the calcium content of the kidney was increased some 25 times, thus reflecting the gross observations. The liver content of calcium was increased six-fold. Average data indicate that the calcium content of the heart was more than doubled on the low magnesium diet but, because of individual variation the increase was not statistically significant. No significance could be attached to a small increase recorded for muscle.

As regards growth rate and general condi-

tion, the results were much more nearly satisfactory when all three minerals were lowered than when magnesium alone was decreased. At autopsy some liver necrosis was observed in all groups but was more severe with the low-magnesium diet. It was found that when the serum magnesium was greatly reduced, the phosphorus was elevated. When the phosphorus was lowered in the same proportions as were the magnesium and calcium (so that the calcium-magnesium, phosphorus-magnesium and calcium-phosphorus ratios remained approximately the same as in the basal purified diet growth) performance was much better than when the magnesium content alone was reduced, but not as satisfactory as that obtained on the unmodified Reid-Briggs or stock diets. However, no calcium deposits were found in the kidneys. In the case of the diet in which magnesium and calcium were lowered, with a resulting high phosphorus-magnesium ratio, the growth rate was less than for the case in which magnesium and phosphorus was lowered, but both of these produced better growth than was observed with a reduction of magnesium alone. Two animals on low magnesium and calcium showed kidney calcification; animals on low phosphorus and magnesium were normal in this respect.

In summary: These data indicate that decreasing the magnesium content of a satisfactory purified diet for guinea pigs results in a lowered serum magnesium, an elevated serum phosphorus and a high degree of kidney calcification, in contrast to the absence of these findings when calcium and phosphorus are proportionately lowered as well. Thus, the deleterious effects of low magnesium are due primarily to an imbalance of mineral relationships.

NOTES

Assay of Catechol-Amines in Urine

The determination of catechol-amines in urine has both academic and diagnostic value. These substances include adrenaline, noradrenaline, dihydroxyphenylalanine, and hydroxytyramine. A rather simple bioassay, using changes in the blood pressure of a rat as an index, has been proposed recently by M. A. Floyer (*Lancet* **II,** *1154 (1958)*).

While it is not claimed that this is a highly precise assay, it is a quick method for screening, using simple apparatus and, according to the authors, requiring little technical skill. The assay probably measures noradrenaline and, hence, would give low results for total catechol-amine in patients excreting a high proportion of adrenaline.

In six individuals who had proven pheochromocytoma, the rat assay conclusively showed increased quantities of pressor-amines in the urine.

In a second paper by J. T. Wright (*Lancet* **II,** *1155 (1958)*) the excretion of catechol-amines in the urine was also studied, using a chemical method. This investigator found that the catecholamines would form complexes with boric acid and, as such, could be extracted from solutions with basic resins. It is believed that the method is simple, quick and economical in operation.

The complex is eluted from the resin column by dilute mineral acids and the catechols are then estimated fluorimetrically. Using this procedure, it is possible to estimate the 24-hour urinary content of both adrenaline and noradrenaline.

Improvements in quantitative methods for the determination of these amines, and the adapting of them to estimate blood levels should aid in the obtaining of more information concerning the intermediary metabolism, and role, of these substances in the general regulation of bodily processes.

Wheat Gluten and Serum Cholesterol

A recent review in this journal (*Nutrition Reviews* **17,** 62 (1959)) calls attention to

conflicting evidence as to the effect of dietary protein on serum cholesterol, and discusses experiments in which soybean meal protein evidently caused diminution in dietary hypercholesterolemia in the chicken.

N. Nath, A. E. Harper and C. A. Elvehjem have studied the effect of dietary protein on serum cholesterol concentrations in the rat. They report in a letter to the editor (*Arch. Biochem. & Biophys.* **77,** 234 (1958)) that wheat gluten protects against experimental hypercholesterolemia in rats.

Their rats were fed a diet containing 25 per cent hydrogenated coconut oil, 1 per cent cholesterol and 0.5 per cent cholic acid. Dietary protein was varied at the expense of sucrose. When the diet contained 6 per cent casein the serum cholesterol was 853 mg. per cent after three weeks; at 40 per cent casein, it was 431 mg. cholesterol per cent, and at 69.5 per cent, it was 561 mg. cholesterol per cent. With wheat gluten ranging from 10 to 68.5 per cent, the serum cholesterol levels ranged progressively from 486 down to 167 mg. per cent. Thus, at each level for which comparisons were made, serum cholesterol values were lower in gluten-fed rats than in those fed casein. The food intake and weight gain correlated inversely with the cholesterol levels and this is, of course, the reverse of the usual cholesterol-lowering effect of underfeeding or clinical malnutrition (*Nutrition Reviews* **12,** 220 (1954)).

However, to show that their results are not due to differences in these factors, the authors fed 30 per cent wheat gluten supplemented with 1 per cent lysine. These rats had much greater weight gain than those given the same diet without lysine, yet had almost the same cholesterol levels (260 and 250 mg. per cent, respectively). Why did the malnourished rats show the highest serum cholesterol levels?

Tryptophan Uptake by Carcinoid Tumors

Several reviews have discussed the formation of 5-hydroxytryptamine (serotonin) by the argentaffin tumors called carcinoids (*Nutrition Reviews* **15,** 156 (1957); **16,** 294 (1958)). One note (*Ibid.* **16,** 223 (1958)) reviews the experiment in which J. W. Cole and L. Matthews gave radioactive tryptophan orally to a patient with a carcinoid tumor in an effort to trace its incorporation into 5-hydroxytryptamine. Biopsy fragments of secondary tumor yielded 10.2 counts per minute per mg. of dry tissue; muscle and liver showed 1.5 and 6.8 c.p.m., and it was concluded that the tumor took up the labeled tryptophan preferentially.

The same authors have been able to study a second similar patient and have reported briefly (*A.M.A. Arch. Surg.* **76,** 912 (1958)). The patient was operated upon for obstruction of the small intestine. A tumor of the terminal ileum and a segment of bowel were resected and a solitary nodule, thought to be a metastatic tumor, was observed in the liver. The primary tumor proved microscopically to be a carcinoid and a second operation was undertaken to remove the liver nodule. Five hours before operation 25 microcuries of C^{14}-labeled tryptophan were given by mouth.

The secondary tumor and fragments of other tissues were removed and assayed for C^{14} activity. The tumor gave 1205 counts per minute per mg. dry weight; skeletal muscle, liver and rectal mucosa yielded 1.0, 2.6 and 5.0 c.p.m., respectively.

Cole and Matthews point out that the tremendous uptake of labeled tryptophan by the tumor in relation to other tissues is consistent with the large amounts of 5-hydroxtryptamine which these tumors contain and make, presumably, from tryptophan.

Perhaps the fact that the tumor was in the liver, exposed to the full concentration of absorbed tryptophan in the portal blood, made the uptake especially striking in this case, but the experiment is all the more definitive, as the liver tissue took up only about 2 per cent as much.

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